Short-term effects of retention forestry on the diversity of root-associated ectomycorrhizal fungi in Sakhalin fir plantations, Hokkaido, Japan

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Introduction

Retention forestry is based on the concept of retaining structural elements in the harvested stands for a long time, at least until next rotation of logging, in order to preserve ecosystem functions with achieving timber production. Ectomycorrhizal fungi (EcMf) are one of the groups of organisms that benefit significantly from tree retention. However we have quite limited views regarding with the effects of retention forestry in East Asian plantations due to scarcity of experimental data from the region and the forest type.

The way of tree retention can be roughly divided into two types, dispersed and aggregated retention, and there is rather unified view on the different effects of the two practices on the conservation of EcMf diversity. Aggregated retention (**Fig. 1C**) is a more efficient way to conserve EcMf diversity than dispersed retention (**Fig. 1F**) because EcMf communities are more influenced by post-harvest environmental filtering, than by the continuity of dispersed trees.

In this study, we aimed to examine the efficacies of dispersed and aggregated retention on conserving EcMf diversity in roots of retained trees and naturally regenerated conifer seedlings in Sakhalin fir (*Abies sachalinensis*) plantations, Hokkaido, Japan.

Materials and methods

Study sites were located at the foot of Mt. Irumukeppu in Hokkaido, Japan (**Fig. 1A**) where large-scale manipulative experiments known as "the Retention Experiment for plantation Forestry in Sorachi, Hokkaido (REFRESH)" are undertaken from 2013 in Sakhalin fir plantations (47–71 years-old, 55 on average). There were seven treatments in the experiment; clear-cutting (CC), three levels of broadleaved dispersed retention (SS, SM and SL), conifer aggregated retention (GR), unharvested Sakhalin fir plantations (PC) and natural broadleaved forests (NC) (**Fig. 1B-H**). A series of treatments were carried out three-times (sets 1 to 3) every other year from 2014 to 2016.

The natural broadleaved forests consisted mainly of *Tilia japonica*, *T. maximowicziana*, *Acer pictum* and *Quercus crispula*. The unharvested Sakhalin fir plantations consisted of mainly *A. sachalinensis* and broadleaved trees such as *Betula* spp. regenerated at rates of 18–28% of the total number of trees. Naturally regenerated broadleaved trees were retained in a distributed manner at three levels, approximately 10, 50 and 100 trees/ha in low (SS), medium (SM) and high (SL) retention levels, in dispersed retention sites. The retained trees mainly consisted of birch (*B. platyphylla* var. *japonica*, *B. ermanii*, and *B. maximowicziana*), followed by *T. japonica* and *Q. crispula*. In aggregated retention sites, a single patch of Sakhalin fir were retained with square shape (60 × 60 m: 0.36 ha) at the center of the logged areas. In all logged sites, Sakhalin fir seedlings are planted in the first year after logging, and weeding is done every year.

At each site, 7-cm square soil cores under two naturally regenerating Sakhalin fir seedlings (**Fig. 1I**) were taken at 15 points several tens meters apart. The two cores with Sakhalin fir seedlings were pooled as a single sample. In dispersed retention sites, points for collecting samples were set within a few meters from retained trees; mainly *Betula* spp. followed by *T. japonica* and *Q. crispula*. In aggregated retention sites, seven points were set within a patch of Sakhalin fir and eight were set from more than a dozen meters away from the forest edge in surrounding clear-cut areas. Sampling were conducted four-years after logging. In one site of aggregation retention (GR3), most of the trees in the retained patch had been fallen by strong winds a year before the survey was conducted, so only data from surrounding clear-cut areas were used for further analysis. In sites of natural broadleaved forests, roots of mature broadleaved trees were preferentially sampled due to low numbers of Sakhalin fir seedling regenerated there.

Root systems were extracted from soil cores and divided into two groups; roots of Sakhalin fir seedlings and adjacent broadleaved or Sakhalin fir trees (**Fig. 1J**). EcMf root tips were morphotyped based on appearance, and one or two root tips of each morphotype from each soil core were subjected to DNA analysis. Internal transcribed spacer (ITS) region (ITS1–5.8S–ITS2) of nrDNA was amplified by PCR with a primer pair of ITS1F and ITS4 and then sanger-sequenced. ITS sequences were submitted to BLAST search to deduce taxonomic lineages of the EcMf. Aligned data matrix of the sequence were subjected to MOTHUR v.1.39 and sequences were grouped into operational taxonomic units (OTUs) based on 97–98% similarity threshold.

The incidence frequency data of OTUs for each site were used for analysis. Data in aggregated retention were divided into two subsets, i.e., inside of retention patches (GRin) and surrounding clear-cut areas (GRout), and then subjected to the analysis. All analysis were performed using R v.4.1.1. Sample-coverage-based rarefaction and extrapolation curves were plotted to compare species diversity among treatments. To see the dissimilarity of EcMf communities among sites, non-metric multidimensional scaling (NMDS) was conducted with the chao dissimilarity index using metaMDS function. In the NMDS, data for each site, except for natural broadleaved forests, were divided into two subsets, i.e., Sakhalin fir seedlings and surrounding retained trees, and then subjected to the analysis separately.

Results and discussion

Clear-cutting significantly reduced the OTU richness and diversity of root-associated EcMf, and alter OTU compositions when compared with unharvested Sakhalin fir plantations and natural broadleaved forest stands (**Fig. 2, 3**). Both aggregated and dispersed retention clearly increased OTU diversity of common and dominant EcMf species (Hill numbers q = 1 and 2) when compared to clear-cut sites, but there was no clear effects in aggregated retention sites and dispersed retention sites of middle retention levels in OTU richness (q = 0, **Fig. 2**), possibly due to high probability to newly encounter with rare EcMf species, even in clear-cut sites. Furthermore, OTU richness in aggregated retention sites and dispersed retention sites tended to be lower than that of the unharvested Sakhalin fir plantations and natural broadleaved forests, respectively (**Fig. 2**), even though both retention methods could retain EcMf diversity that would otherwise be lost by clear-cutting (**Fig. 3A**). The results suggest that the both retention methods of the present study had positive but to some degree limited effects in conserving the diversity and species richness of EcMf.

NMDS ordination plots indicated that EcMf compositions in the retention patches of conifer aggregated retention sites were similar with that of unharvested Sakhalin fir plantation, while those of surrounding logged areas were similar with those of clear-cut sites (**Fig. 3A**). Also, OTU richness was clearly higher in the retention patches than in the surrounding logged areas (**Fig. 2B**). The results indicated that aggregated retention is effective in conserving EcMf diversity of unharvested Sakhalin fir plantations but the effects were limited within patches and were negligible in the surrounding logged areas. Furthermore, coverage-based inter- and extrapolation curves indicated that OTU richness in the retention patches was clearly lower than unharvested Sakhalin fir plantations (**Fig. 2B**). It was likely that EcMf diversity in the retention patches of the small size (0.36 ha in this study) can be affected by edge effects caused by logging the surrounding areas, which lead to decrease the OTU richness in the retention patches.

EcMf communities were similar among sites of dispersed retention irrespective of retention levels, and different from either those in Sakhalin fir plantations, natural broadleaved forests and also clear-cut sites (**Fig. 3A**). The results indicated that broadleaved dispersed retention can not retain well EcMf diversity of the forests before logging even in high retention level possibly due to environmental filtering but can retain unique EcMf diversity in conifer plantations. Also, OTU richness in some dispersed retention sites were comparable to that of unharvested Sakhalin fir plantations, and higher than that of retention patches in aggregated retention sites (**Fig. 2**). It indicated that dispersed retention is not necessarily inferior to aggregated retention when it comes to preserving a larger number of EcMf species and retain unique EcMf diversity in logged sites of conifer plantations, although the positive effects can be spatially limited within rooting zones of the retained broadleaved trees.

Regenerating Sakhalin fir seedlings shared 56% and 51% of OTUs with surrounding broadleaved tress in dispersed retention sites and with surrounding Sakhalin fir trees in aggregated retention sites, respectively. Moreover, NMDS ordination plots clearly showed that OTU compositions were relatively similar between Sakhalin fir seedlings and their surrounding trees (**Fig. 3B**). It is well known that proximity of trees of different species affects the community compositions of EcMf, although host identity is likely the primary factors that select their symbionts. Such neighborhood effects may be one of the factors that increase the rate of shared OTUs and similarity of OTU compositions between Sakhalin fir seedlings and surrounding broadleaved trees in the retention sites. Further studies are needed to understand the effects of the common mycorrhizal networks on the growth of surrounding Sakhalin fir seedlings in logged areas.

Our findings demonstrated that both dispersed and aggregated retention can mitigate the impacts of logging on EcMf diversity in Sakhalin fir plantations, in Japan, but there are large differences in the effects among the different retention methods.

Fig. 1A: Location of study sites in Hokkaido, Japan. **B**: Clear-cut site. **C**: Conifer aggregated retention site. **D-F**: Broadleaved dispersed retention sites at low (D), middle (E) and high retention level (F). **G**: Unharvested Sakhalin fir plantation. **H**: Natural broadleaved forest. **I**: A Sakhalin fir seedling. **J**: Ectomycorrhizal roots of a broadleaved tree (arrow) and Sakhalin fir (triangle). Roots of Sakhalin fir are clearly wider than those of broadleaved trees and easily distinguishable from them.

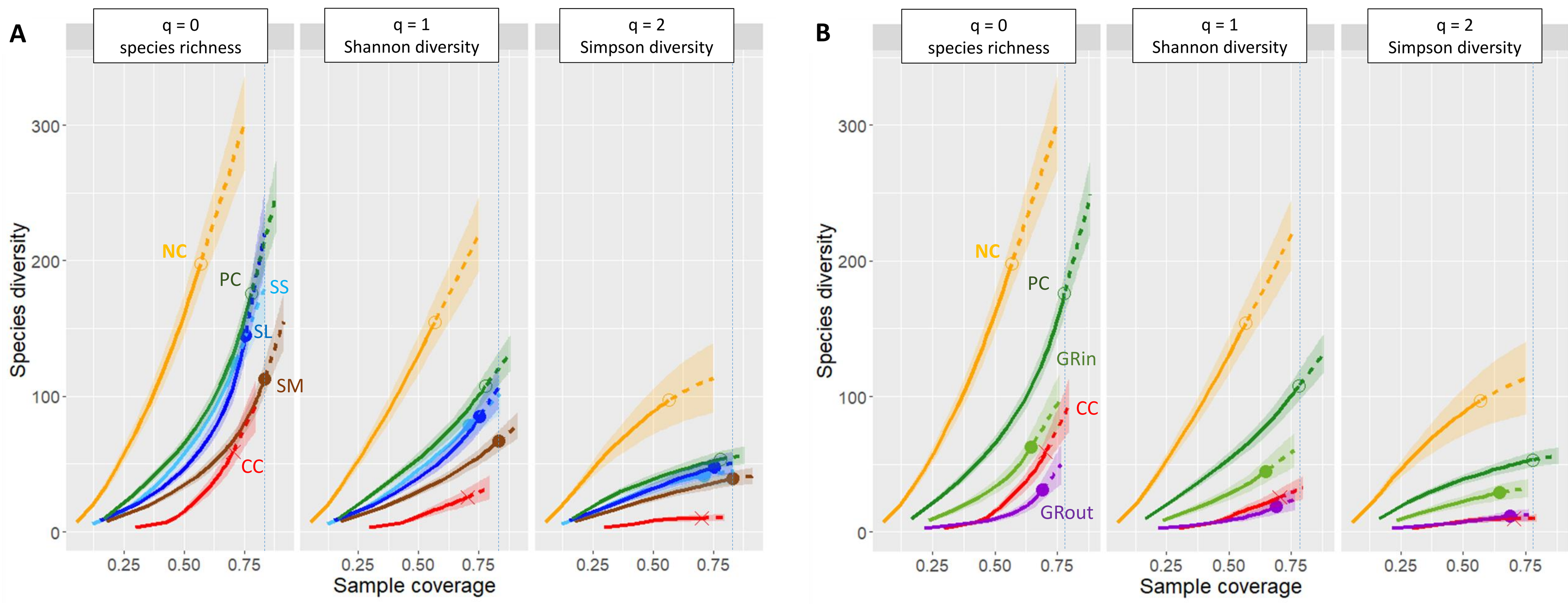

Fig. 2: Coverage-based intra- and extrapolation curves of each treatment for the Hill number of orders q = 0 (species richness), q = 1 (the exponential of Shannon entropy) and q = 2 (the inverse of Simpson concentration), which can be interpreted as indicating the effective number of all, common and dominant species, respectively. Curves of dispersed retention (**A**) and aggregated retention sites (**B**) were indicated with control treatments (CC, PC and NC). Sample coverage is a measure of sample completeness, giving the proportion of the total number of individuals in a community that belong to the species represented in the sample. Shaded areas indicated 95 % confidence intervals based on 1,000 bootstrap replications. Species diversity is considered to be clearly different among treatments when the confidence intervals do not overlap each other at the equal value of sample coverage (blue dotted lines).

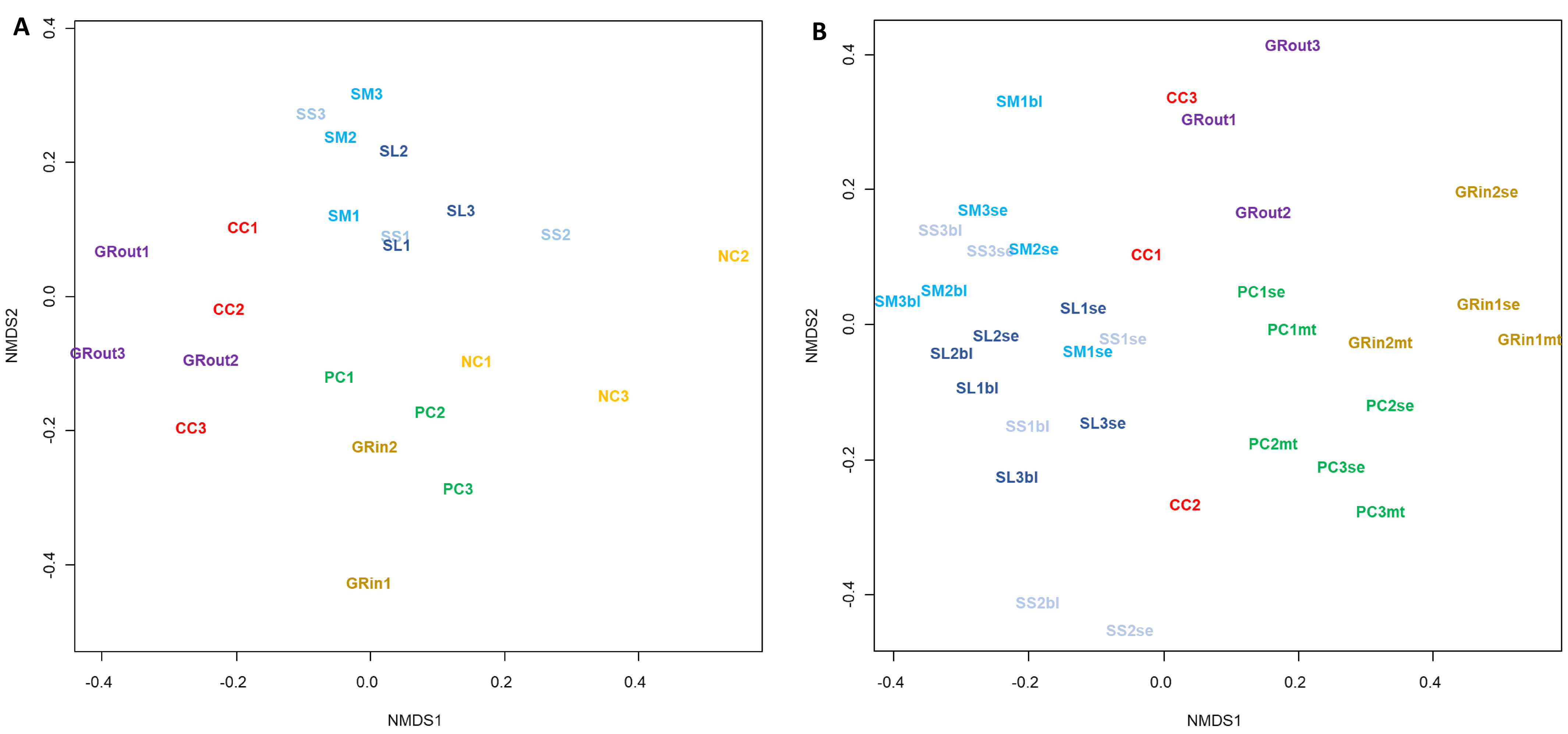

Fig. 3: NMDS ordination plots illustrating dissimilarities of OTU compositions using chao dissimilarity index among study sites (**A**) and among study sites with different origins (**B**); Sakhalin fir seedlings (SITE+"se", e.g., SS1se), surrounding Sakhalin fir (+"mt", e.g., GRin1mt) or broadleaved trees (+"bl", e.g., SS1bl). Data within retention patches (GRin) and surrounding logged areas (GRout) in GR sites were separately subjected to the analysis.

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